

Figure 1A.

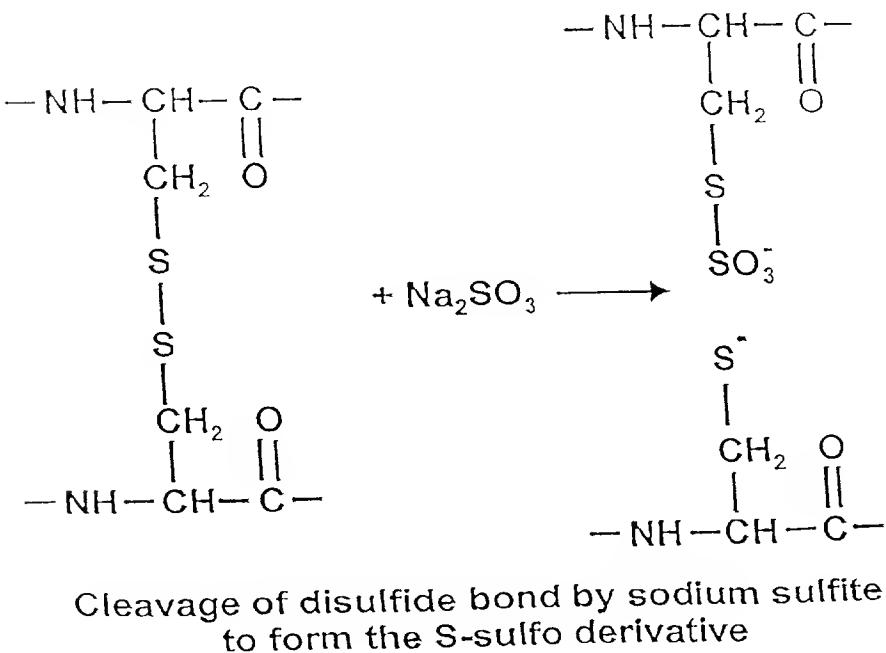


Figure 1B.

Preparation and Washing of TnI-containing Inclusion Bodies

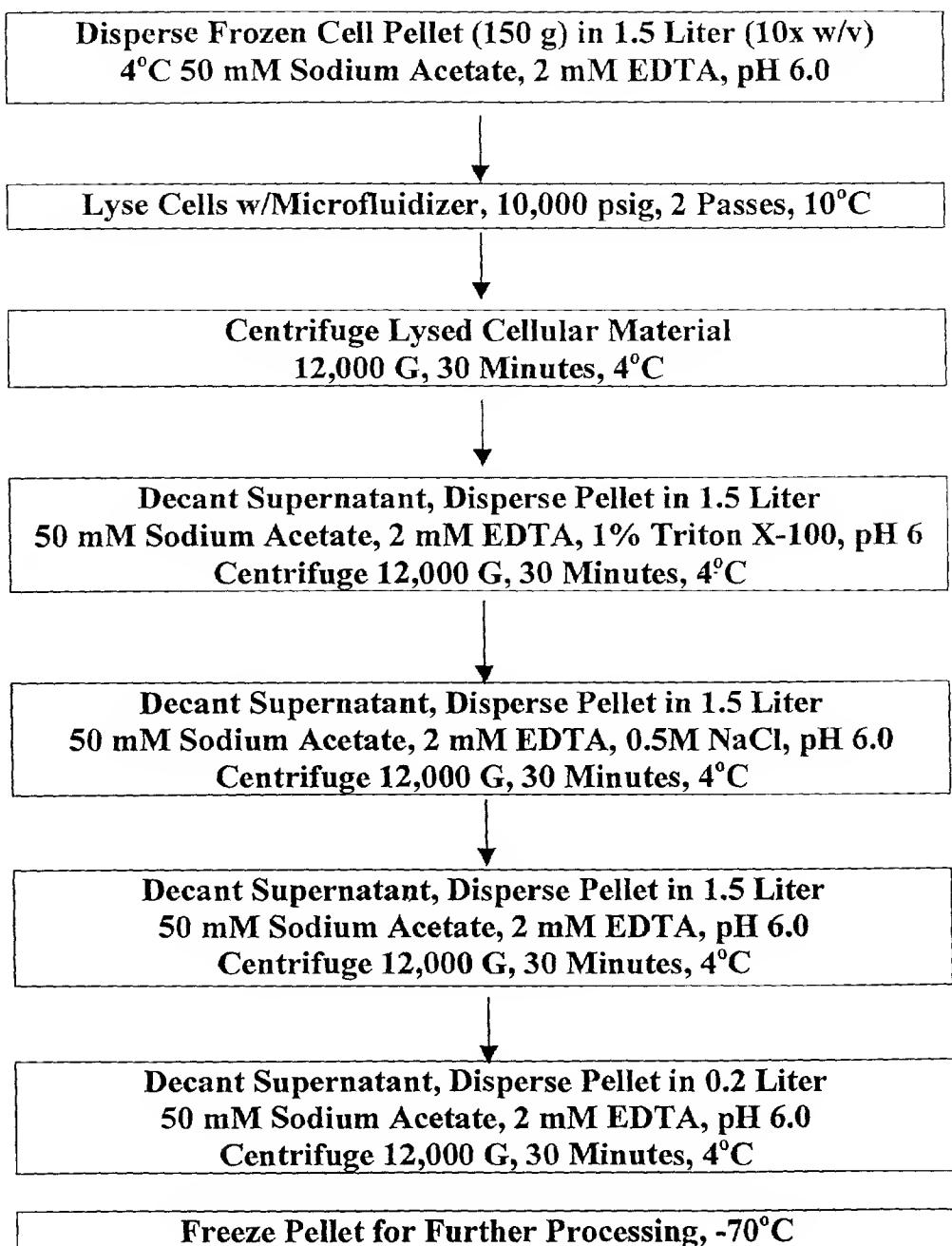


Figure 2

Summary of rTroponin-I Preparation – 3L5 (050900-051200)

3L Q-Sepharose FF column.

3L Q-Sepharose FF column.

Buffer A: 6M urea, 25mM Tris-HCl, pH 7.5, 100mM NaCl.

Buffer B: 6M urea, 25mM Tris-HCl, pH 7.5, 2M NaCl.

Gradient: Step; 0% B for the flow-through then to 100% B for strip and cleaning.

Flow rate: 150ml/min.



UF/DF; 0.2 ft² Pall Omega cassette.

- 3000 ml applied – 10-fold concentration (UF) to 300 ml.
- Buffer exchange (DF) against 5L 6M urea, 25mM Tris-HCl, pH 7.5.
- 290 ml as the final retentate volume...load for 300 ml Q-Sepharose FF column.



300 ml Q-Sepharose FF column.

Buffer A: 6M urea, 25mM Tris-HCl, pH 7.5.

Buffer B: 6M urea, 25mM Tris-HCl, pH 7.5, 2M NaCl.

Gradient: Step; 4% B for elution and 50% for cleaning.

Flow rate: 20ml/min.



60 ml Toyopearl 650M HIC (phenyl) column.

Buffer A: 6M urea, 25mM Tris-HCl, pH 7.5, 1M (NH₄)₂SO₄.

Buffer B: 6M urea, 25mM Tris-HCl, pH 7.5.

Gradient: Step; 100% B for flow-through to 0% B for strip and cleaning.

Flow rate: 10ml/min.



UF/DF; 0.2 ft² Pall Omega cassette.

- 550 ml applied
- Buffer exchange (DF) against 5L 25mM citrate, pH 3.0, 150mM NaCl.
- 200 ml as the final retentate volume
- 100, 1 ml aliquots and 25, 4 ml aliquots.
- Final 3L5 product.

Figure 3

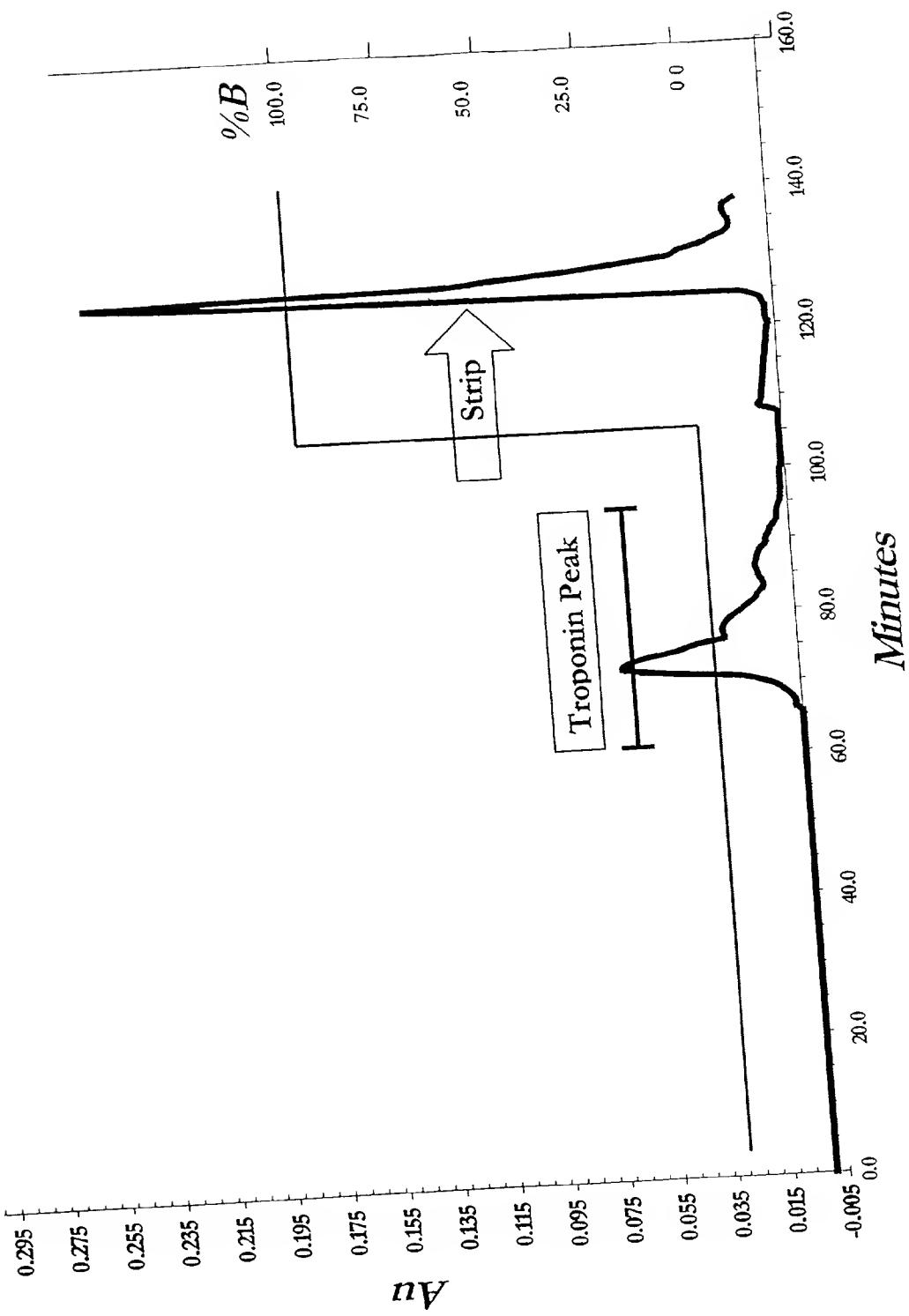


Figure 4

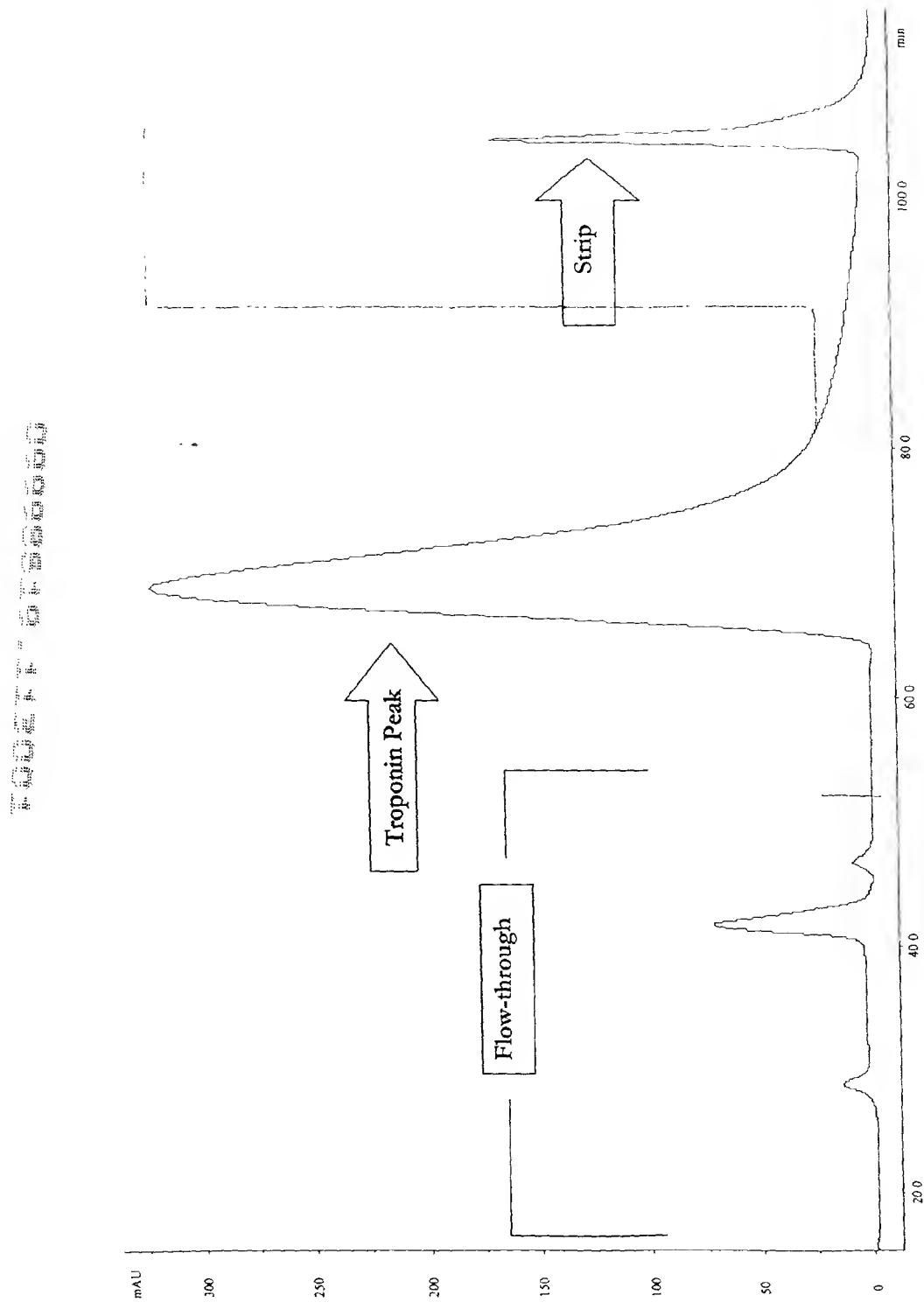


Figure 5

SDS-PAGE Analysis Trop onin Lot 3L5

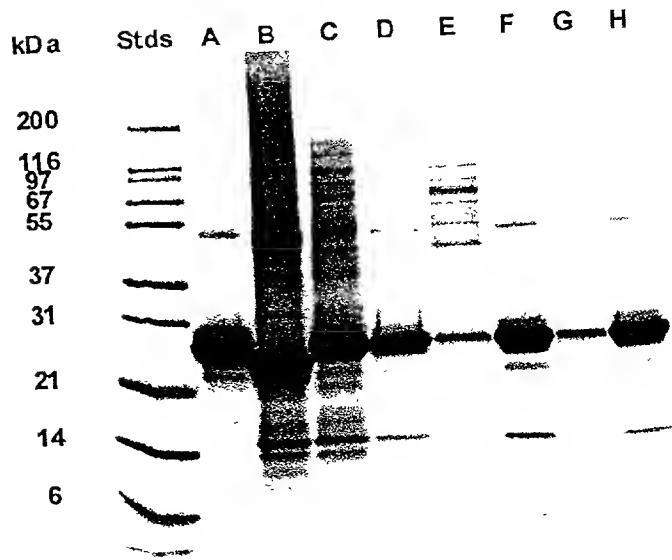


Figure 6

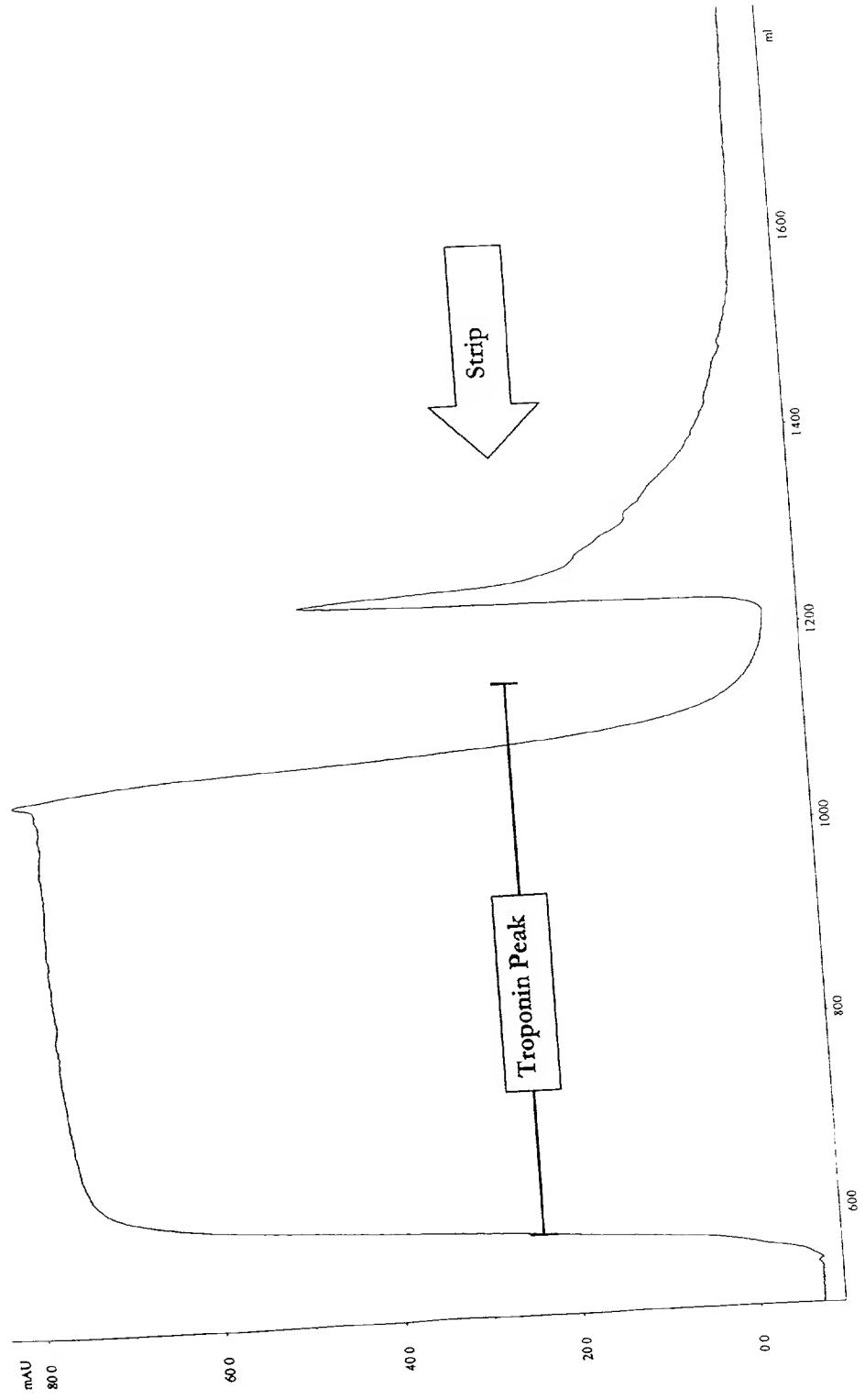


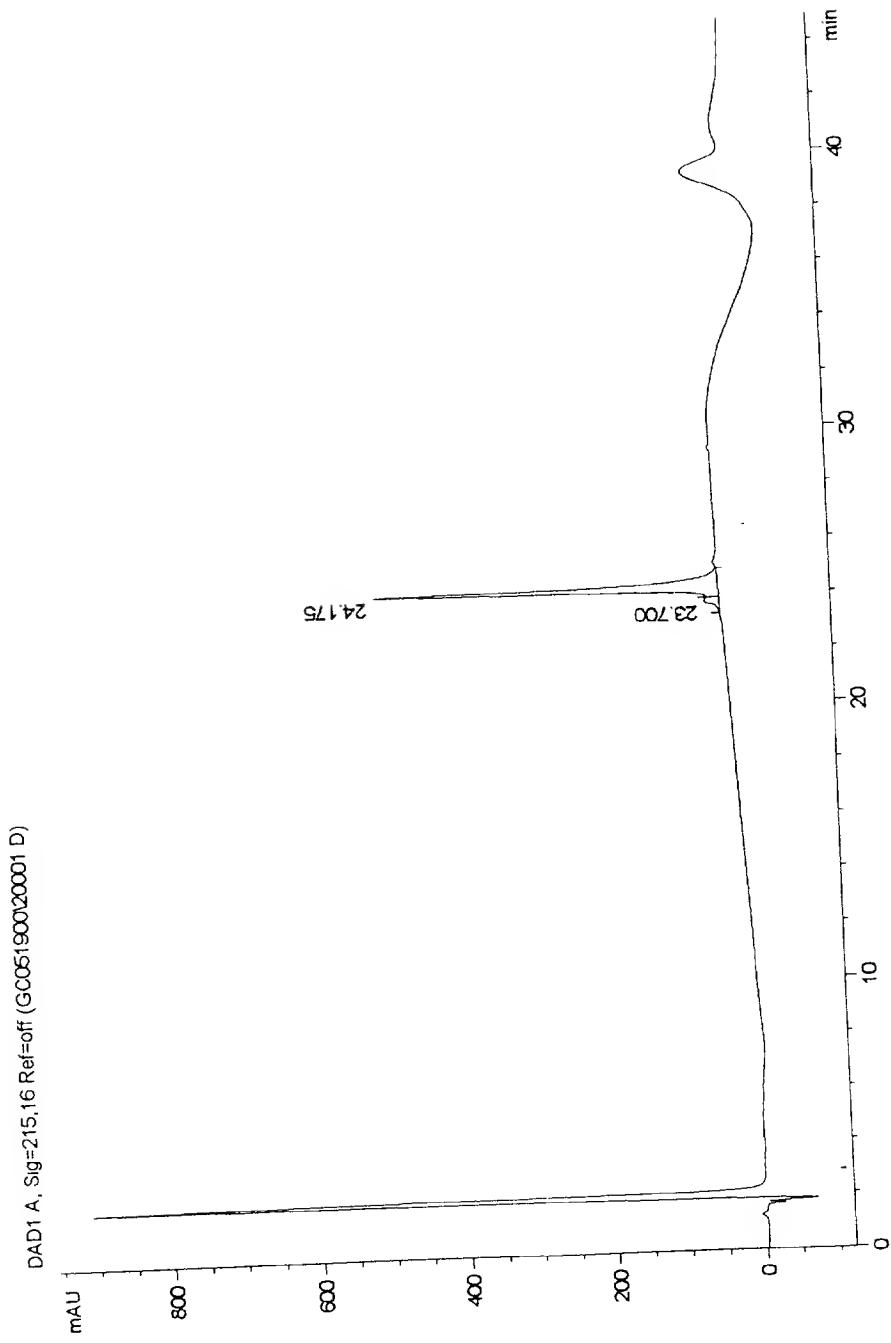
Figure 7

SDS-PAGE Analysis Troponin Lot 3L5
Hydrophobic Interaction Chromatography
16% Tris-glycine Gel, Non-reducing, 5/15/00



Figure 8

Figure 9



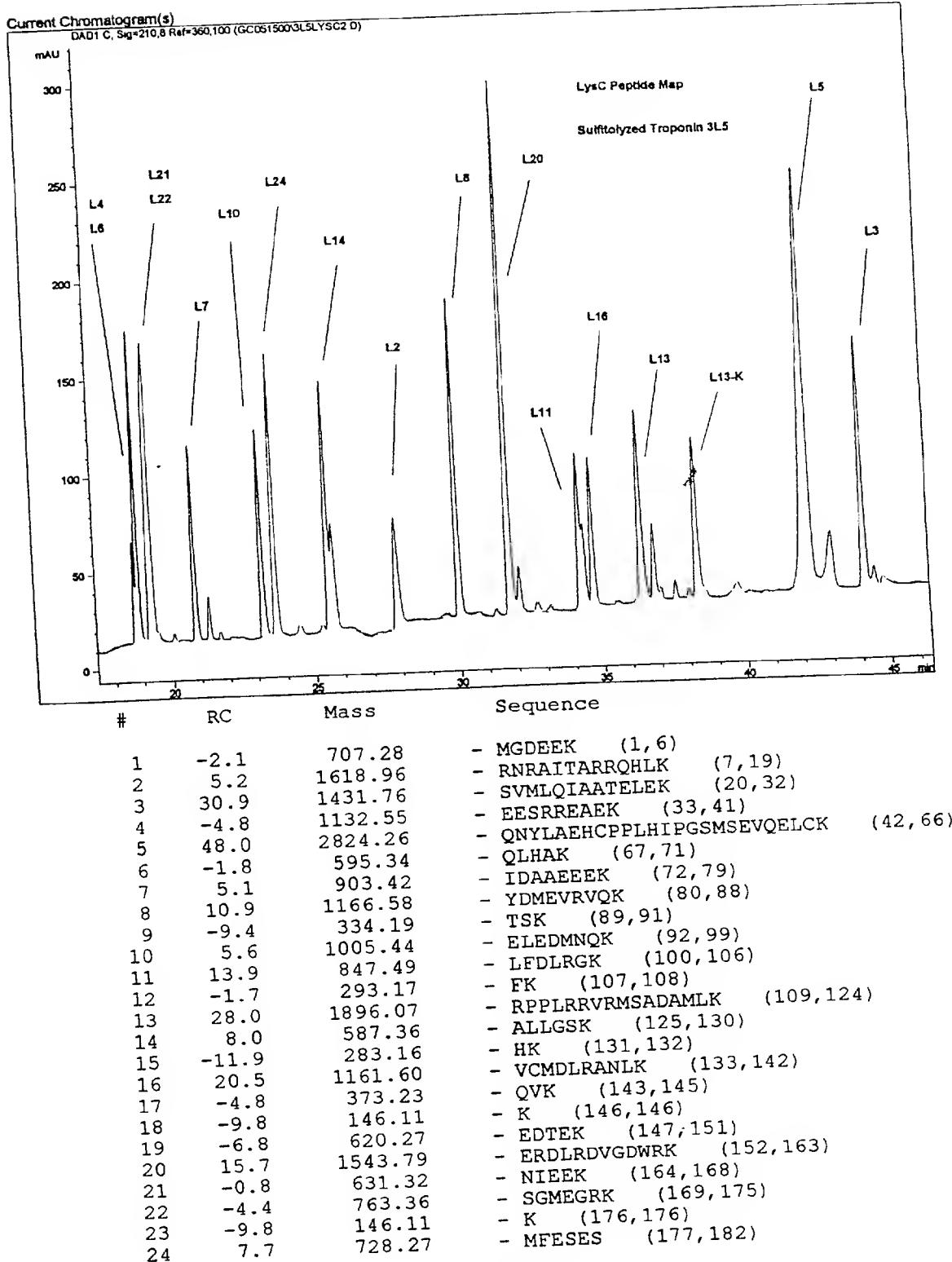


Figure 10

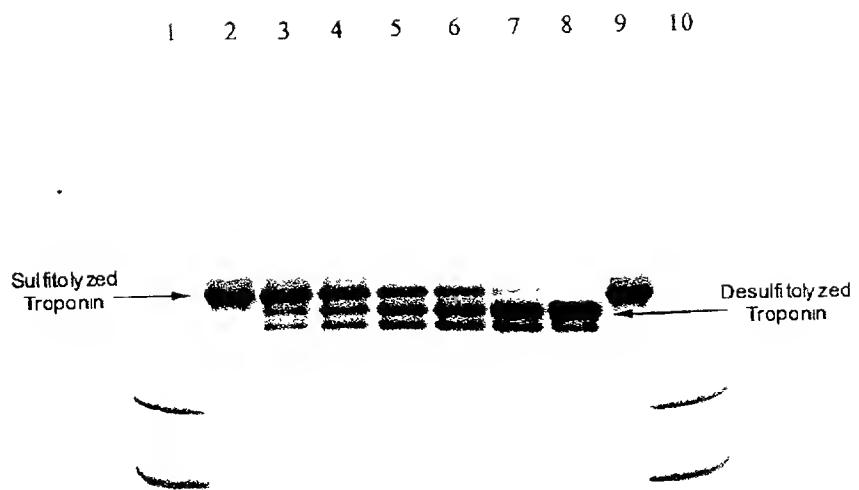


Figure 11